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Direct Analysis of Carbofuran and Its Carbamate Metabolites in Rapeseed Plants by Nitrogen–Phosphorus Detector Gas Chromatography

Young W. Lee and Neil D. Westcott*

Carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran were extracted from acid-digested rapeseed plants with methylene chloride. The concentrated methylene chloride extract was passed through a column that contained carbon–attaclay and silica gel. The eluant of this column was concentrated and passed through a column containing acid alumina, Florisil, and silica gel. The fractions containing carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran were collected and concentrated separately prior to the addition of the internal standard. Carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran were determined by gas chromatography with a nitrogen–phosphorus detector using a 15% Apiezon L column. Recoveries of carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran averaged 85% for all three compounds in the 0.03–5-ppm range. The residues of carbofuran in rapeseed plants were maximum in the plants which were collected 6–8 days after seeding. The concentration of the metabolites, 3-ketocarbofuran, and 3-hydroxycarbofuran increased until 11 days after seeding.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is widely used for controlling flea beetles (mainly *Phyllotreta* sp.) on rapeseed crops in Western Canada. Flea beetles may severely damage a rapeseed crop from the time the seedlings emerge until the crop is established in the field a few weeks later. Carbofuran is applied either as an in-furrow granular or a foliar spray treatment to control flea beetle feeding on seedling rapeseed crops. To develop an understanding of how granular carbofuran protects young rapeseed plants, it was imperative that a method be developed to determine the persistence of carbofuran and two of its main metabolites in rapeseed plants.

Carbofuran and its main metabolite, 3-hydroxycarbofuran, have been determined by gas chromatography (GC) either directly or by prior derivatization. Direct analysis of carbofuran and 3-hydroxycarbofuran has been carried out on corn utilizing microcoulometric detection and a 20% SE-30 column (Cook et al., 1969). Williams and Brown (1973) applied a similar method to the analysis of the same two compounds in small fruits but used an electrolytic detector and a 6% OV-210 and 4% OV-101 mixed-phase column.

An analytical method was developed for carbofuran, nonconjugated 3-hydroxycarbofuran, and 3-ketocarbofuran (Figure 1) using a silica column and a mobile phase of trimethylpentane–2-propanol in high-pressure liquid chromatography (HPLC) with an ultraviolet (UV) adsorption detector (Lawrence and Leduc, 1977). Recently Lee and Westcott (1980) developed an analytical method for carbofuran and 3-hydroxycarbofuran in rapeseed plants by reverse-phase HPLC.

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Residues of carbamate insecticides and their metabolites in various crops have been analyzed by several methods (Bowman and Beroza, 1967; Butler and McDonough, 1971; Cassil et al., 1969; Van Middelem et al., 1971). Apparently there are no previous publications on direct quantitative analysis of carbofuran and its carbamate metabolites, 3-ketocarbofuran and 3-hydroxycarbofuran, in rapeseed plants by GC using a nitrogen–phosphorus detector (N–P detector).

This paper describes a direct analytical method for carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran in rapeseed plants by GC using a N–P detector and a nonpolar, nonsilicon liquid phase, Apiezon L. Metalaxyl (Figure 1) was used as an internal standard in this study. The method was used to determine carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran in rapeseed plants that grew in plots treated with granular carbofuran.

EXPERIMENTAL SECTION

Apparatus. The GC was a Hewlett-Packard Model 5730A equipped with dual N–P detectors (Model 18789A). The column was a 2 mm i.d. × 1.2 m glass column packed with 15% Apiezon L on Chromosorb W (acid washed, dimethyldichlorosilane treated). The operating conditions were column, injector, and detector temperature 170, 200, and 300 °C, respectively. The carrier gas, helium, flow rate was 30 mL/min and hydrogen and air flow rates were 3 and 50 mL/min, respectively. The sensitivity settings were electrometer range 1 and attenuator 16. The recorder was a Perkin-Elmer Model 56 and chart speed was 5 mm/min. The homogenizer was a Virtis, Model 45.

Reagents. All organic solvents were glass-distilled residue-free grade, and the water was distilled in a metal Barnsted still. Carbofuran (99.5% purity), 3-hydroxycarbofuran (99% purity), and 3-ketocarbofuran (analytical standard) were obtained from FMC Corp., Agricultural

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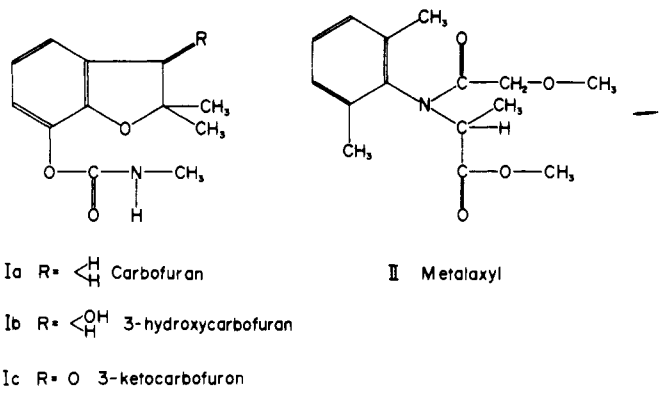

Figure 1. Chemical structures of carbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran, and metalaxyl.

Chemical Division, Middleport, NY, and were dissolved in benzene–ethyl acetate (1:1 v/v). A formulated product containing metalaxyl [methyl *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-2-aminopropionate] was obtained from Ciba-Geigy Canada, Ltd., Etobicoke, Ontario, Canada. The crude metalaxyl was extracted with methylene chloride, and the extract was concentrated, placed on a Florisil column, and eluted with benzene–hexane (1:1 v/v). The eluant was evaporated to dryness. The residue was recrystallized from hexane. The melting point of the purified metalaxyl was 73–74 °C (uncorrected). This metalaxyl was dissolved in benzene–ethyl acetate (1:1 v/v) for use as the internal standard. In addition, the following reagents were all used as received: sodium lauryl sulfate, reagent grade; sodium chloride, ACS reagent grade; silica gel, Davidson grade 923, 100–200 mesh; hydrochloric acid, ACS reagent; decolorizing carbon, Nuchar S-N (all from Fisher Scientific Co.); attaclay (Minerals and Chemicals Division, Engelhard Minerals and Chemicals Corp., Attapulgus, GA); acid alumina (ICN Pharmaceutical Inc., Cleveland, OH). Florisil (Floridin Co., Berkerly Springs, WV) was activated at 130 °C for 5 h before using. The keeper solution was 0.4% paraffin oil (Fisher Scientific Co.) in ethyl acetate.

Field Plot Treatment. Field experiments were carried out from May to June in 1977 in Saskatoon, Saskatchewan, Canada. Rapeseed (*Brassica campestris* L. var. Torch) was planted in randomized split plots, 2.44 m × 4.88 m, with two replicates on May 27. The seeding rate was equivalent to 5.6 kg/ha with carbofuran, formulated as Furadan 5G, being placed in furrow at time of seeding at the rate of 280 and 560 g of active ingredient (a.i.)/ha. Sufficient rape seedlings had emerged by June 2 for the first sample to be collected; subsequent samples were collected June 4, 7, 10, 17, and 24. Samples of rapeseed plants were cut off at ground level, chopped into pieces less than 1 cm long with clean scissors, transferred to plastic bags, sealed, and frozen at −20 °C until analyzed.

Procedure. The steps in the analysis of carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran are shown in Figure 2.

Extraction. Chopped rapeseed plants (20 g) in a 500-mL blender cup containing H_2O (100 mL) were homogenized at medium speed for 2 min. The macerate was transferred to a 1-L round-bottom flask with the aid of H_2O (150 mL), and 12 N HCl (5.2 mL) was added. The acidified macerate was refluxed by using an Allihn condenser and stirred magnetically for 1 h before being cooled to room temperature. After being cooled the condenser was washed down with 0.25 N HCl (50 mL) and removed. The blender cup and blade assembly were rinsed with CH_2Cl_2 (50 mL), and the rinsings were added to the acid-digested macerate. The macerate was cooled in a

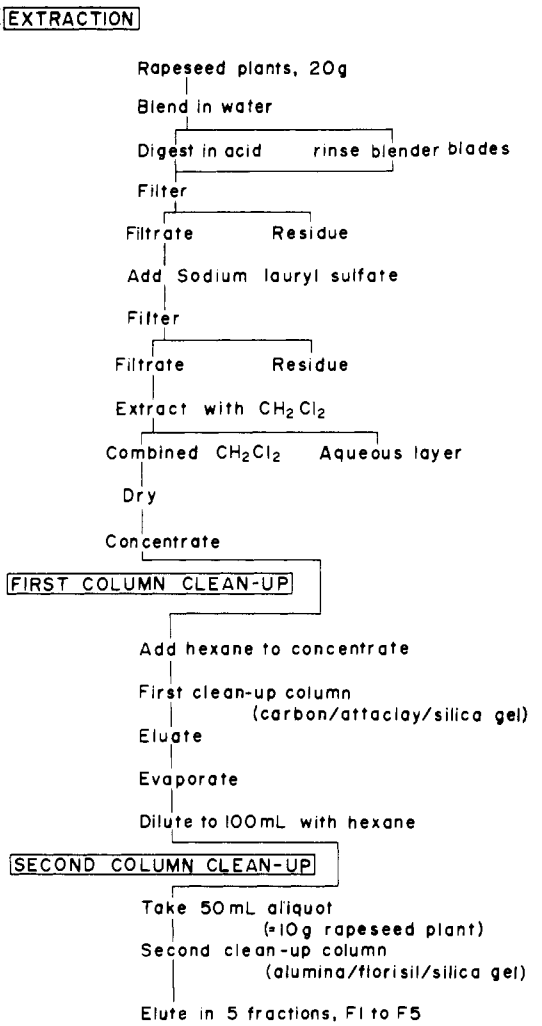

Figure 2. Scheme of analysis of carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran residues in rapeseed plants.

freezer for 1 h or until ice just started to form or placed in the refrigerator (4 °C) overnight. The macerate was filtered through a glass wool pad on a Büchner funnel with suction, and the solids were rinsed with 0.25 N HCl (50 mL) and then with CH_2Cl_2 (2 × 25 mL). Sodium lauryl sulfate (10 mL of a 4% aqueous solution) was added to the filtrate and mixed well, and the mixture was filtered through filter paper on a Büchner funnel with suction. The precipitate was washed with 0.25 N HCl (50 mL) and CH_2Cl_2 (50 mL) with the washings being added to the filtrate. The filtrate was transferred to a 1-L separatory funnel and extracted 3 times with CH_2Cl_2 (50, 100, and 100 mL) with vigorous shaking for 2 min each time. The combined CH_2Cl_2 extract was dried over Na_2SO_4 (40 g) and filtered through filter paper on a Büchner funnel into a 1-L round-bottom flask. The drying flask and Na_2SO_4 were washed 3 times with CH_2Cl_2 (3 × 25 mL) and filtered into the round-bottom flask. The combined CH_2Cl_2 extract was concentrated to 30 mL on a rotary vacuum evaporator at a bath temperature of <25 °C (all evaporations were done in this manner). Hexane (20 mL) was added to the concentrated extract.

First Column Cleanup. To a column (2.2-cm i.d.) with a glass wool plug at the bottom was added ethyl acetate (50 mL) and Na_2SO_4 (10 g). Before the column was packed, carbon (0.4 g) and attaclay (6.6 g) were mixed well and washed with ethyl acetate (2 × 60 mL). This carbon–attaclay mixture in an ethyl acetate slurry was added to the column and packed tightly with suction. The wall of the column was rinsed with ethyl acetate, then silica gel

(5 g) which had been washed with ethyl acetate (2 × 30 mL) was added as an ethyl acetate slurry to the column above the carbon–attaclay with suction, and the sides of column were rinsed with ethyl acetate. The column was capped with a plug of glass wool. The solvent was drained from the column with suction until the level of ethyl acetate was even with the top of the glass wool cap in the column. CH_2Cl_2–hexane (50 mL, 1:1 v/v) was added and drained with suction to the top of the glass wool cap and the eluate was discarded. The receiver was changed, the dried and concentrated rapeseed plant extract was added to the column, and the collection of the eluate was started immediately with suction. The extract was drained to the top of the silica gel, the flask and the wall of the column were rinsed with CH_2Cl_2–hexane (5 × 10 mL, 1:1 v/v), the solvent was drained to the top of the silica gel, and then the column was eluted with hexane–ethyl acetate (250 mL, 1:4 v/v); this fraction was concentrated to 10 mL on a rotary evaporator and quantitatively transferred to a 100-mL volumetric flask and made up to the volume with hexane.

Second Column Cleanup. Anhydrous sodium sulfate was added to a depth of 1 cm over a glass wool plug in a column (1.5-cm i.d.) containing C_6H_6–hexane (50 mL, 1:4 v/v). Acid alumina (10.0 g, without solvent, dry packing) was added to the column. The wall of the column was washed down with C_6H_6–hexane (1:4 v/v). A 1-cm layer of Na_2SO_4, Florisil (10.0 g in C_6H_6–hexane, slurry packing), a 1-cm layer of Na_2SO_4, silica gel (10.0 g in C_6H_6–hexane, slurry packing), and a 1-cm layer of Na_2SO_4 were added to the column in order bottom to top. The column was drained until the level of solvent was even with the top of the Na_2SO_4, and the eluate was discarded. A 50-mL aliquot (representating 10 g of tissue) of the sample from the first column was added to the second column and drained to the top of the Na_2SO_4. The flask and the wall of the column were rinsed with ethyl acetate–hexane (2 × 10 mL, 1:9 v/v) and then eluted with C_6H_6–hexane (100 mL, 1:4 v/v). This constitutes fraction 1 (F1) and was discarded. The receiver was changed and the elution was continued with ethyl acetate–hexane (300 mL, 1:4 v/v) to give fraction 2 (F2). This fraction was normally discarded, but before being discarded it was analyzed for carborfuran contents. Occasionally this fraction contained some carbofuran depending on carbofuran concentration in the sample. The receiver was changed and the column was eluted with ethyl acetate–hexane (300 mL, 3:7 v/v) to give fraction 3 (F3) which contained carbofuran and 3-ketocarbofuran. The receiver was changed, and the column was eluted with ethyl acetate–hexane (150 mL, 2:3 v/v) to give fraction 4 (F4) which was normally discarded. The receiver was changed and the column was eluted with ethyl acetate–hexane (300 mL, 4:1 v/v) to give fraction 5 (F5) which contains 3-hydroxycarbofuran. To fractions 2, 3, and 5 was added 1 mL of keeper solution, and the fractions were evaporated separately to 0.3–2 mL. The internal standard was added and an aliquot was injected into the GC.

Calculations. The concentrations of the three carbamates were determined via the internal standard method based on peak areas as measured by a Hewlett-Packard 3352 laboratory data system. A response factor for carbofuran, f_c, was determined from chromatographing a synthetic mixture of the three compounds plus the internal standard, metalaxyl, by using eq 1

$$f_c = (W_i/W_c)/(A_c/A_i) \quad (1)$$

where W_i and W_c are the weights of the internal standard and weight of carbofuran present in the synthetic mixture, respectively, and A_i and A_c are the areas of the internal standard and carbofuran peaks on the chromatogram, respectively.

Before an unknown sample was chromatographed a known weight, in micrograms, of the internal standard was added, W'_i, to the partially evaporated eluate from the second cleanup column and the sample mixture was chromatographed under the identical conditions as were used for determination of the response factor. The peak areas for carbofuran, A'_c, and for the internal standard, A'_i, were measured from the chromatograms of the sample mixture. The concentration of carbofuran, C_c, expressed as ppm, in the original plant material was calculated from

$$C_c = (W'_i/f_c)(A'_c/A'_i)(1/G) \quad (2)$$

where G is the equivalent weight of plant material in grams taken for the second cleanup column.

The response factors and concentrations for 3-keto- and 3-hydroxycarbofuran were determined in the same manner.

RESULTS AND DISCUSSION

In the operation of a N–P detector, several processes can affect the performance of the detector. The collector, the alkali source, will be adversely affected by chemical coating of the source and loss of the salt. Coating of the source with silicon dioxide from either silicon liquid phase or silanizing reagents is of major concern since it is not possible to remove the silicon dioxide with any solvent that would not damage the collector. As the silicon dioxide layer on the collector increases, high collector voltages are needed which can cause loss of the alkali salt, as well as a decrease in nitrogen selectivity and sensitivity and an increase in noise level. Collector lifetime can be lengthened by using well-conditioned lightly coated silicon liquid phases or by using nonsilicon based liquid phases. In the present study, none of the silicon-based liquid phases when used at low loading ratios provided adequate separations and for that reason Apiezon L was used as the liquid phase.

The linearity of detector response to carbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran, and metalaxyl was checked over the range of 0.5–7.0 ng for carbofuran, 2.8–33.6 ng for 3-ketocarbofuran, 2.6–31.0 ng for 3-hydroxycarbofuran, and 2.9–34.8 ng for metalaxyl per injection. The detector responses were linear for all four compounds in the range studied. Carbofuran was the most sensitive; 3-ketocarbofuran, 3-hydroxycarbofuran, and metalaxyl were about equally sensitive but less than carbofuran. The minimum detectable concentrations were at least 0.03 ppm for all three carbamates in a 10-g sample of rapeseed plants. A new standard solution was made every 2 weeks for 3-ketocarbofuran as it decomposed with time.

Incorporation of the acid hydrolysis step (Cook et al., 1969) in the extraction procedure allows the determination of both nonconjugated and conjugated metabolites of carbofuran. The ability to determine the conjugated metabolites is important in view of the work of Dorough (1968) and Knaak et al. (1970), who found up to 97% of metabolites were conjugated. When the acid-digested rapeseed samples were extracted with methylene chloride, some samples formed emulsions which took a very long time to separate. So that this could be overcome, 10–30 mL of saturated NaCl aqueous solution in 5-mL portions was added and shaken. If too little or too much saturated sodium chloride solution was added, it did not break the emulsion.

The amount of Nuchar decolorizing carbon was a critical feature of the cleanup procedure. It was found that some commercial decolorizing carbon–attaclay mixtures reduced

Table I. Recovery of Carbofuran, 3-Ketocarbofuran, and 3-Hydroxycarbofuran from 10 g of Fortified Rapeseed Plants

fortification, ppm			recovery, %,[a] ± SE[b]		
carbofuran	3-ketocarbofuran	3-hydroxycarbofuran	carbofuran	3-ketocarbofuran	3-hydroxycarbofuran
5.000	5.600	5.160	90.9 ± 1.2	91.1 ± 2.8	88.3 ± 4.0
1.000	1.120	1.032	92.9 ± 3.5	91.5 ± 2.2	96.2 ± 1.7
0.100	0.112	0.103	80.0 ± 4.9	89.3 ± 5.0	87.4 ± 3.7
0.030	0.034	0.031	76.1 ± 5.1	71.2 ± 4.7	72.8 ± 3.3
			av: 85.0	85.8	86.2

[a] Results are the mean percentage recovery from three replicates. [b] SE, standard error.

Table II. Residues of Carbofuran, 3-Ketocarbofuran, and 3-Hydroxycarbofuran from Two Treatment Rates in Rapeseed Plants

		residues, ppm, for a rate of application of carbofuran of					
		280 g of a.i./ha			560 g of a.i./ha		
sampling date	days after seeding[a]	carbofuran	3-ketocarbofuran	3-hydroxycarbofuran	carbofuran	3-ketocarbofuran	3-hydroxycarbofuran
June 2	6	2.01	0.23	0.79	2.25	0.33	1.29
June 4	8	1.36	0.34	1.47	2.96	0.79	3.19
June 7	11	1.19	0.39	2.27	2.25	0.73	6.48
June 10	14	0.24	0.13	1.73	0.39	0.25	2.32
June 17	21	0.07	0.11	0.54	0.15	0.03	0.55
June 24	28	trace[c]	ND[b]	0.20	ND	ND	0.44

[a] Seeded May 27. [b] ND, not detectable. [c] Trace amounts: less than 0.03 ppm of carbofuran.

the recovery efficiency. In preparing our own decolorizing carbon–attaclay mixture, it was found that the amounts of decolorizing carbon and attaclay described under Experimental Section were optimal for both cleanup purposes and maintaining acceptable recovery efficiencies.

The second cleanup column separated carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran from numerous coextractives. Fraction 1 had mainly the waxy compounds from rapeseed plants; occasionally fraction 2 had some carbofuran depending on the concentration of carbofuran in the sample. Keeper solution was added to fraction 2 and evaporated to 0.3–2 mL prior to addition of internal standard and injection into the GC to determine the carbofuran contents. Fraction 2 had some extraneous peaks, one of which is close to 3-ketocarbofuran and may interfere with quantitation of 3-ketocarbofuran which is eluted in fraction 3 with carbofuran. Fraction 4 had extraneous peaks, one of which occurred near the 3-hydroxycarbofuran peak which was collected in fraction 5. The materials for the cleanup column were accurately weighed on an analytical balance and the elution solvents measured by using pipets and volumetric flasks. This two-column cleanup procedure provided purified extracts of rapeseed plants that could be quantitatively analyzed by the N–P detector. Figure 3 shows the chromatograms of the various fractions and the separation and resolutions obtained. The retention times of carbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran, and the internal standard (metalaxyl) were 9.4, 11.9, 15.9, and 18.1 min, respectively.

The recoveries obtained by using spiked rapeseed plant samples are given in Table I. The recovery efficiencies obtained were reasonable and consistent across the range studied. Recoveries of carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran from laboratory-fortified samples ranged from 71 to 96% with an average of 85% for all three compounds in the range of 0.03–5 ppm.

All rapeseed plant samples from each field plot were analyzed in duplicate, and average values from the two plots are reported in Table II but are not corrected for recovery. The blank rapeseed plant samples which were collected from both untreated field-grown and greenhouse-grown plants did not show any interfering peaks at the retention times of carbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran, and the internal standard, metalaxyl.

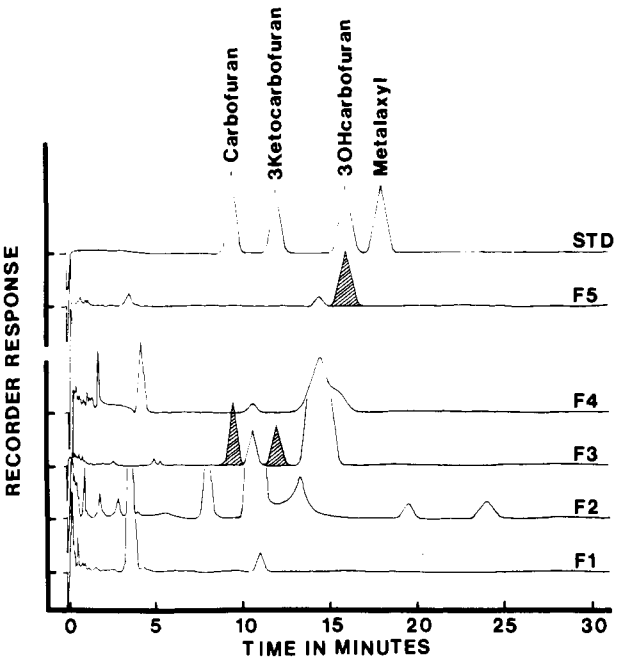

Figure 3. Chromatogram of the standard solution (STD) and fractions 1–5 (F1–F5) from the second cleanup column of blank and spiked rapeseed plants. Top chromatogram (STD): 2-μL injection of carbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran, and metalaxyl standard solution (2.00, 5.08, 5.36, and 5.80 μg/mL, respectively). Bottom chromatogram (F1–F5): Injections of fractions 1–5 of the second cleanup column of blank rapeseed plants and plants spiked at the 0.1-ppm level with carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran.

The concentrations of carbofuran and its conjugated and nonconjugated metabolites in rapeseed plants grown in soil that received the high rates of carbofuran were higher than those grown in soil receiving the low rate of carbofuran. The pattern of change in residue concentration of carbofuran or metabolites was essentially independent of the

rate of application of the granular carbofuran. The residues of carbofuran were at a maximum concentration 6–8 days after seeding and then decreased quickly between 11 and 14 days after seeding. By 28 days after seeding, the residues of carbofuran were trace or not detectable. The concentrations of a 3-ketocarbofuran residues were always lower than those of either carbofuran or 3-hydroxycarbofuran. The highest concentrations of 3-ketocarbofuran, 0.8 ppm, occurred in the period 8–11 days after seeding in plants collected from the plots receiving the high treatment rate of carbofuran. Concentrations of 3-ketocarbofuran decreased after the period so that by 28 days after seeding residues of 3-ketocarbofuran were not detectable.

The maximum concentration of 3-hydroxycarbofuran residues, conjugated and nonconjugated, were found in the samples collected 11 days after seeding with 6.48 ppm found in plants grown with the high rate of treatment. The concentration of 3-hydroxycarbofuran decreased throughout the remainder of this study so that residues of 0.20 and 0.44 ppm were detected in plants grown in plots treated at the low and high rate, respectively, 28 days after seeding. The relatively high level of 3-hydroxycarbofuran compared to that of carbofuran and 3-ketocarbofuran is likely the result of the rapeseed plants conjugating this metabolite, thus slowing its degradation (Knaak et al., 1970).

The detection of carbofuran and its metabolites in emerging seedlings of rapeseed indicates that absorption of carbofuran by the seedlings starts prior to emergence, thus providing chemical protection against flea beetle feeding.

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Extraction of Biologically Incorporated [^{14}C]Carbofuran Residues from Root Crops

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[^{14}C]Carbofuran, suspended in a commercial formulation, was added to the soil and exposed roots of growing potatoes, carrots, and radishes. The treated crops were harvested at 5, 10, and 15 days post-application and composited. Carbofuran residues in the composites were extracted by blending with methanol or acetonitrile, followed by washing and leaching with the same solvent used in the blending. With potatoes and radishes, both solvents extracted approximately equal percentages of [^{14}C]carbofuran residues (85% for potatoes and 37% for radishes from 15-day samples) for all harvest-time combinations. With carrots, methanol extracted more ^{14}C than acetonitrile for all harvest-time combinations (e.g., 95.8 vs. 90.7% from the 15-day sample). Methanol was as efficient as acetonitrile for extracting ^{14}C from radishes and potatoes. Residues extracted from samples with methanol or acetonitrile were partitioned into methylene chloride and characterized. Carbofuran was the major residue in potatoes and radishes; the level decreased with longer incorporation periods. The angelic acid ester of 3-hydroxycarbofuran, carbofuran, and 3-hydroxycarbofuran were the main residues in carrots. The aqueous phase of each extractant, after partitioning with methylene chloride, contained a significant quantity of ^{14}C, which increased with incorporation period. Acid hydrolysis of the aqueous phase released the following residues as major aglycons of conjugated carbofuran metabolites: 3-keto-7-phenol for potatoes, 3-hydroxycarbofuran for carrots, and an unidentified metabolite for radishes. The crop marc remaining after Soxhlet extraction contained a significant amount of bound residues (up to 62.1% of total) only in radishes. Acid hydrolysis of these crop marcs released some bound residues into aqueous solution, but only a minute quantity of ^{14}C could subsequently be extracted into methylene chloride.

The introduction and increasing use of carbamate pesticides have resulted in a need for analytical methodology which can be used to determine carbamate residues in foods and environmental samples. Analytical methods for determining multiple carbamate residues in samples are highly desirable. These methods must provide efficient extraction of residues from the sample matrix.

The extraction of pesticide residues can be evaluated by using samples in which radioactively labeled pesticides are biologically incorporated. This allows the determination of extracted and unextracted residues; conjugated and bound pesticide residues can also be measured. Although

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